REMARKS

On April 22, 2003 the undersigned attorney had a telephonic discussion with Examiner Davis regarding the Office Action issued on January 30, 2003 and the lack of examination of applicants' elected product claim. On October 25, 2002, applicants filed a response to the imposed restriction requirement that was issued on September 25, 2002, wherein applicants chose to prosecute the invention of Group 29, which included **product** claim 12. Applicants have included, as evidence, a copy of October 25, 2002 response in Appendix B, wherein applicants stated, at page 6, that they provisionally elected with traverse, the invention of Group 29 (claim 12) for further examination on the merits. Applicants further stated in accordance with guidelines recited in MPEP Section 821.04, that when the elected product claim was found patentable, they intended to rejoin the method claims.

Notwithstanding, applicants' election of **product claim 12** for further prosecution, the Office mistakenly examined the method claims 1-4, 8, 9, 23 and 24. Further, the Office withdrew from further consideration claims 5-7, and 10-11 but did not mention the current status of claims 12-22. During the April 22, 2003 telephonic discussion with Examiner Davis, it was suggested that applicants remind the Office of this oversight and the Office Action of January 30, 2003 would be vacated and reissued. However, to move prosecution forward, applicants are requesting that Examiner Davis examine product claim 12 and other recited mutations in SEQ ID NO. 2 and consider the following remarks applicable for determining the patentability of the above-identified method claims that applicants intend to rejoin upon finding the product claims allowable. Further, applicants are requesting that they are given sufficient time to provide a response to the next issued Office Action without the Office issuing the next Office Action as a Final.

It should be noted that Applicants were the first to discover the calcium-binding protein "calmyrin" and its interaction with presenilin 1 and 2. The protein was identified as "calmyrin" because the name describes its inherent properties without bias towards its multiple binding partners. Applicants discuss at page 20 of the specification the process used to discover the calmyrin protein (SEQ ID NO: 2) and that it was named by its interaction with the PS2-loop B bait construct. As stated in the specification, applicants named the calcium binding protein "calmyrin" because is was shorter than using the complete terminology "calcium-binding myristoylated protein with homology to calcineurin." Further, applicants discovered that calmyrin was ubiquitously expressed in all tissues that were examined which implies that it plays a common function in most if not all cells.

Once this protein was discovered by applicants and the protein-protein interaction with the presenilins was determined, the next step in the process was to determine how to reduce the affinity between the presenilins and calmyrin thereby altering the cell death cascade and reduce the effects of cell death, especially in neuronal cells of familiar Alzheimer Disease (FAD) patients. Applicants set about, as shown with the multiple experiments set forth in the specification, to determine effective mutations in the calmyrin (SEQ ID NO 2) and presenilin 2 (SEQ ID NO: 1) that would reduce the affinity of calmyrin for presenilin 2 or at least modulate the interaction between the two proteins so that the normal triggering of the cell death cascade would be averted or at least reduced.

Applicants discovered several mutations to be effective in reducing the affinity of calmyrin for presenilin 2 and/or modulating the interaction between the two proteins. These substitution mutations in calmyrin include:

1) substituting at least one amino acid residue in the calcium-binding EF-hands of SEQ ID NO: 2, wherein the calcium-binding hands including amino acid residues at positions 116 to 128 and 161 to 173 of SEQ ID NO: 2;

2) substituting at least one N-terminal residue at position 1 to 3 of SEQ ID NO: 2; and

3) substituting at least one amino acid residue at position 2, 127 and/or 172 of SEQ ID NO: 2.

All of these mutations are clearly defined in the specification. For example, at page 33 there is a discussion as to how the nucleotide sequence of calmyrin (SEQ ID NO: 26) was altered to introduce the mutations at certain amino acid residues, including amino acid residues 2, 127 or 172. The mutated calmyrin was then tested for affinity with presenilin 2 and it was found that there was reduced affinity between the proteins and as a result reduced cell death was evident.

Further, it should be noted that the discovery of this calmyrin protein provides for the advantage of using the calmyrin protein in assays to determine other compounds that inhibit the interaction of the calmyrin with presenilin 2. Also, antibodies can be raised against the calmyrin protein and used to bind to the calmyrin and reduce protein-protein interaction with presenilin 2.

Applicants clearly showed that mutation of the calmyrin protein had the effect of reducing protein-protein interaction and further provided a step by step detailed description in the specification for using this mutated calmyrin protein. For example, as discussed on pages 44- 48 of the specification, there are

multiple uses of the calmyrin protein, either in an unmutated state or mutated state to modulate cell death.

In the January 30, 2003 Office Action, method claims 1-4, 8-9 and 24 were rejected under 35 U.S.C. §112, second paragraph. Although applicants elected to have the product claim 12 examined before the method claims, applicants have amended independent method claims 1 and 24 thereby obviating this rejection.

Method claims 1-4, 8-9 and 23-24 were rejected under 35 U.S.C. §112, first paragraph because, according to the Office, "the specification, while being enabling for a method for reducing apoptosis *in vitro*, does not reasonably provide enablement for a method for inducing apoptosis *in vivo*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims." Applicants insist that to be enabling, the specification must simply set forth "a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same. Applicants have met this standard.

The Office speculates that there is no correlation between familiar Alzheimer Disease (AD) and induced apoptosis caused by the interaction between presenilin 2 and calmyrin. Applicants vigorously disagree and submit that it is well established that presenilin 2 is implicated in the regulation of programmed cell death (see page 3 of present application) and causes the majority of early onset of familiar Alzheimer's disease. Applicants discovered a calcium-binding partner (calmyrin) of the expressed presenilin proteins and have further shown that the protein-protein interaction caused an increase in apoptosis. Further, by mutating specific regions of the newly discovered binding partner calmyrin, the protein-protein interaction is reduced and apoptosis is reduced. A reasonably skilled molecular biologist would clearly be capable of following the steps set forth in the specification that would permit one of skill in the art to make and use the claimed invention.

According to the Office, *in vitro* conditions of Hela cells cannot represent complex *in vivo* conditions and cultured cell lines generally differ significantly from the characteristics of a primary tumor. Initially, it should be noted that applicants used a carefully regulated set of experiments using Hela cells that are customarily used for screening of testing compounds for potential use in human. Hela

cells have been used in studies investigating drugs and other compounds because there is a clear correlation between results found *in vitro* testing using Hela cells and *in vivo* testing models. For example, Navarova, et al. (Life Sciences, 65 (18-19): 1905-1907, Oct. 1999; copy of abstract in Appendix A) determined that the testing results of the cardioprotective drug stobadine were comparable between *in vitro* results found in Hela cells and *in vivo* results in mice. Further, Macickova, et al. (General Physiology and Biophysics, 18: 86-91, Oct. 1999; copy of abstract in Appendix A) determined that induced lysosmal enzyme activity was comparable between *in vivo* results determined in rats and *in vitro* results found in Hela cells. Likewise, Okubo, et al. (AATEX 1, 2-9. Nov. 1999; copy of abstract in Appendix A) showed *in vitro* cytotoxicity testing results in Hela cells were comparable to *in vivo* results in rabbits thereby providing an alternative testing regime to replace the *in vivo* Draize eye irritation test used to test cosmetics and other compounds. Nobuyuki, et al. (Environmental Health Perspectives, 102, 3, Sept. 1994; copy of abstract in Appendix A) determined similar correlation between toxicity induced by chromate found in an *in vivo* testing model and *in vitro* results in Hela cells. Still further, Ookata, et al. (Biochemistry, 36 (50): 15873-15883, Dec. 1997; copy of abstract in Appendix A) determined that the same phosphorylation sites were found in both *in vitro* testing using Hela cells and *in vivo* tests. All of the above references show a strong correlation between the results of *in vitro* testing in Hela cells and *in vivo* results and clearly contradict the references cited by the Office.

The references cited by the Office discuss cell cultures but a primary or secondary cell culture cannot be compared to immortalized cells such as Hela cells which unlike primary or secondary cells, continue to grow and divide indefinitely *in vitro* for as long as the correct culture conditions are maintained. Hela cells are the classic example of an immortalized cell line and are adherent cells and not oncogenic in animals, unless transformed by a virus. Specifically, the Drexler, et al. reference, cited by the Office, states at page 3, that the Hodgkin's disease HD cell lines used in the studies were not immortalized cells, and thus, cannot be compared to immortalized Hela cells. Embleton, et al., also cited by the Office, discusses the lack of antigens on cultured cells thereby reducing the accuracy of interpreting results obtained with monoclonal antibodies but the present invention does not include the production of antibodies for the Hela cells or tumor cells but instead antibodies are raised for epitopes on calmyrin. Thus, the Embleton, et al reference is not relevant to the presently claimed invention. The Office further cites Freshney that discusses the disadvantages of *in vitro* cell cultures and states that cellular metabolism may be more constant in *in vitro* than *in vivo*. However, it must be recognized

by the Office that Freshney further states that any inconsistency in the cell cultures can be rectified by inclusion of a number of different hormones in the culture media.

Hsu, also cited by the Office, discusses different analysis for monitoring cell population by the chromosome constitution of the *in vitro* cell line. However, applicants do not even consider the chromosome constitution of the cultured cells as being relevant to the present invention, and instead, as clearly stated in the present application, monitor the level of apoptosis by a simple procedure that can include manual counting of dead cells. Clearly, none of the references cited by the Office provide any definitive proof that *in vitro* and *in vivo* test results are not comparable. Likewise, the Office has provided no proof that the overexpression of calmyrin, mutated calmyrin or presenilin altered the cultured cells or its colonization behavior.

In contrast, the references cited by applicants show that there is positive correlation between *in vitro* and *in vivo* testing results. The Board of Patent Appeals and Interference addressed this issue of correlation between *in vitro and in vivo* testing results in *Ex Parte Balzarine*, 21 U.S.P.Q.2d 1892 (BPAI 1991) wherein the examiner cited a 1987 reference by Yarchoan, et al. as proof that there was no correlation between *in vitro* and *in vivo* testing results. However, the Board stated that the appellant could provide evidence that established that those skilled in the art would accept that *in vitro* testing would be useful in *in vivo* treatment of humans. In the present case, applicants have met this burden and presented ample proof of a positive correlation between *in vitro* and *in vivo* testing results, as recognized by those skilled in the art.

According to the Office, the specification does not disclose the domain of the calcium binding protein necessary for binding to presenilin 2. However, a precise location of the binding site is not necessary to establish enablement, since it is not necessary for the ordinarily skilled artisan to know the precise location in order to make and use the claimed invention. It is enough that when the calmyrin is mutated in the disclosed regions, the binding affinity is reduced and in fact the level of apoptosis is reduced.

Further, the Office contends that the decrease in apoptosis is not predictable because the mechanism of cell death level is not known and the mutation in calmyrin cannot be predicted to be the region necessary for binding to presenilin 2. Applicants vigorously disagrees because "[It] is not a requirement for patentability that the inventor know how or why an invention works." *Newman v. Quigg*, 11 USPQ2d 1340 (Fed. Cir. 1989). Furthermore, statements that a physiological phenomenon

(in this case protein-protein interaction) was observed is not inherently suspect simple because the underlying basis for the observation cannot be explained. Statements made by applicants that a mutated calmyrin binding to presenilin 2 reduces apoptosis do not run counter to generally accepted norms. The Office has not provided any evidence that one of ordinary skill in the art would doubt the objective truth of the statements contained in applicants' disclosure.

According to the Office, "the scope of the claims includes a method for reducing apoptosis using numerous structural variants. Applicants have not shown how to make and use the claimed variants which are capable of functioning as that which is being disclosed." In response, applicants stress that it is well settled that patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art. Instead, there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the invention. Applicants have met this standard, by discussing the exact regions for mutations in the calmyrin protein that have been found effective to reduce binding affinity with the presenilins. Specifically, as stated above, these regions include: the calcium-binding EF-hands of SEQ ID NO: 2, including amino acid residues at positions 116 to 128 and 161 to 173; and the N-terminal of SEQ ID NO: 2. Mutating the calcium binding EF hands of calmyrin, discovered by the homology to calcineurin B, reduces the binding affinity between the calmyrin and presenilins thereby reducing apoptosis. One skilled in the art can easily cause a mutation in either the calcium-binding EF-hands or at the 1-3 amino acid residues at the N-terminal of calmyrin and thus practice the presently claimed invention.

The claims, as now amended, recite applicants' invention in terms fully supported in the disclosure defining the subject matter sought to be patented. The claims thus are in compliance with the enablement and written description requirements of the first paragraph of section 112, and withdrawal of the rejections is respectfully requested.

Fees Payable

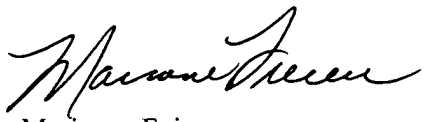
Applicants have added two new independent claims and six new dependent claims beyond the number for which a fee has previously been paid, resulting in an added claim fee of $156.00. Applicants have included herewith a check in the amount of $156.00 for eight additional claims, two of which are independent. The U.S. Patent and Trademark Office is hereby authorized to

charge any additional amount necessary to the entry of this amendment, and to credit any excess payment, to Deposit Account No. 08-3284 of Intellectual Property/Technology Law.

Conclusion

Applicants have satisfied the requirements for patentability. All pending claims are free of the art and fully comply with the requirements of 35 U.S.C. §112. It therefore is requested that Examiner Davis examine the product claim 12 and reconsider the patentability of method claims 1-2, 4-7 and 9- 32 in light of the distinguishing remarks herein and withdraw all rejections, thereby placing the application in condition for allowance. Notice of the same is earnestly solicited. In the event that any issues remain, Examiner Davis is requested to contact the undersigned attorney at (919) 419-9350 to resolve same.

Respectfully submitted,



Marianne Fuierer
Attorney for the Applicants
Registration No. 39,983

INTELLECTUAL PROPERTY/
Technology Law
P.O. Box 14329
Research Triangle Park, NC 27709
Telephone: (919) 419-9350
Facsimile: (919) 419-9354
IPTL File: 4115-161